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Lipoteichoic Acid Is the Major Cell Wall Component Responsible for Surface Hydrophobicity of Group A Streptococci

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The contribution of lipoteichoic acid (LTA) to the hydrophobic surface properties of group A streptococci was investigated in aqueous dextran-polyethylene glycol two-phase systems. Enzymatic digestions were performed to characterize the hydrophobic surface structure. The results obtained indicated that LTA is a major factor responsible for the hydrophobic character of the cell surface of group A streptococci. This was further supported by the similarity of partition in polymer two-phase systems between whole group A streptococci and tritiated LTA extracted from a group A streptococcal strain. Surface LTA was also determined on intact organisms by a new method measuring the adsorption of antibodies to LTA to the bacterial surface. A correlation was found between the content of surface LTA and the hydrophobicity of the group A streptococci. We conclude that surface-associated LTA is the major factor determining surface hydrophobicity of group A streptococci.

Surface properties of bacteria are important in conferring or modulating adherence properties of the bacteria to host cells (20, 28, 32–34, 38, 39). The surface properties of a microorganism will therefore determine the outcome of a host-parasite interaction (31). Adherence of group A streptococci to epithelial surfaces is recognized as a prerequisite for the virulence of these organisms (9). Previous studies have demonstrated that the hydrophobic character of bacteria plays a central role in their interaction with mammalian cells (20, 28, 32, 38, 39). Group A streptococci are known to have a strong tendency to hydrophobic interaction (35). A relationship between M protein and the hydrophobic properties of these bacteria has previously been suggested (24, 35).

Lipoteichoic acid (LTA), another cell wall component of group A streptococci, has been suggested to play a central role in the pathogenesis of streptococcal infections (18, 46). LTA is an amphiphatic molecule composed of 1,3-phosphodiester-linked glycerophosphate and a small lipid moiety (46). Teichoic acids are exposed on the surface of intact bacteria (8, 21, 27, 37). Wicken and Knox have proposed that LTA may be transient within the cell wall (46). The hydrophobic lipid portion of LTA influences the immunogenicity of teichoic acid (45) and is essential for the binding of teichoic acids to cell membranes (7, 26).

In the present investigations, surface structures of group A streptococci responsible for hydrophobicity were further characterized. Partition in aqueous polyethylene glycol (PEG)-dextran two-phase systems containing hydrophobic groups covalently bound to PEG was used to study the hydrophobic surface properties of the bacterial cells (1, 2, 11, 24, 40, 41, 43). LTA extracted from group A streptococci showed a hydrophobic interaction liability similar to that of whole organisms. The hydrophobic affinity of group A streptococci as revealed by partition in polymer two-phase systems correlated to the content of surface LTA. The results indicated that LTA rather than M protein contributes to the hydrophobic affinity of group A streptococci.

MATERIALS AND METHODS

Bacterial strains. A total of 56 bacterial strains were included in the study. The bacterial species and number of strains were as follows: group A streptococci, 34; group C streptococci, 5; group G streptococci, 6; *Klebsiella pneumoniae*, 3; *Pseudomonas aeruginosa*, 4; and *Escherichia coli*, 4. The strains were kindly provided by J. Rotta, Prague, Czechoslovakia (28 strains), and L. W. Wannamaker, Minneapolis, Minn. (2 strains), or were obtained consecutively from clinical specimens sent to the Clinical Microbiology Laboratory, University Hospital, Lund, Sweden. Strains were stored at −90°C in Todd-Hewitt broth supplemented with fetal calf serum and on blood agar plates at 4°C. Bacterial strains were inoculated in Todd-Hewitt broth and incubated for 15 to 17.5 h at 37°C. Bacteria were harvested by centrifugation at 3,500 rpm for 10 min and washed twice in phosphate-buffered

TABLE 1. Effects of enzymatic treatment and heat treatment of group A streptococci on the partition of the bacteria in polymer two-phase systems

Bacterial strain	CP[a]						
	PBS control	Trypsin[b]	Pepsin[c]		Papain[d]	Hyaluronidase[e]	Heat[f]
			pH 4.5	pH 5.8			
AW4	65	14	40	72	24	72	62
AM1	48	10	26	42	17	62	52
AM18	75	12	28	72	26	92	75
AM23	78	16	35	78	26	77	81

[a] See the text.
[b] A total of 150 μg of trypsin per ml, pH 7.2.
[c] A total of 100 μg of pepsin per ml.
[d] A total of 200 μg of papain per ml, pH 8.0.
[e] A total of 50 U of hyaluronidase per ml, pH 7.2.
[f] A temperature of 100°C for 10 min.

saline (PBS; 0.03 M sodium phosphate, 0.12 M NaCl, pH 7.2). The optical density at 620 nm was measured, and the bacterial concentration was calculated from a standard curve and adjusted to 10^9 organisms per ml.

Radiolabeling of bacteria. For bacterial radiolabeling, [^{3}H]glycerol, [^{3}H]thymidine, ^{51}Cr (sodium chromate), and ^{125}I (sodium iodide) (Radiochemical Centre, Amersham, England) were used. Bacterial labeling with ^{125}I was performed by the following procedure. A total of 10^9 bacterial organisms were suspended in 200 μl of PBS. Then 15 μCi of ^{125}I, 5 μl of 0.015% hydrogen peroxide in PBS, and 5 μl of lactoperoxidase (Sigma Chemical Co., St. Louis, Mo.) were added to the cell suspension and incubated at room temperature for 10 min. The bacteria were deposited by centrifugation, washed four times in ice-cold PBS, and suspended in PBS to 5.0×10^8 organisms per ml. Alternative labeling of bacteria with ^{51}Cr was performed as previously described (24). Internal labeling with [^{3}H]thymidine was performed by growing bacteria in Todd-Hewitt broth containing 2 μCi of [*methyl*-^{3}H]thymidine per ml (specific activity, 25 Ci/mmol; Radiochemical Centre). Cells were harvested, washed four times, and suspended in PBS to 10^9 organisms per ml. LTA was radiolabeled by growing the bacteria in Todd-Hewitt broth supplemented with 1.5 μCi of [2-^{3}H]glycerol (specific activity, 500 mCi/mmol) per ml.

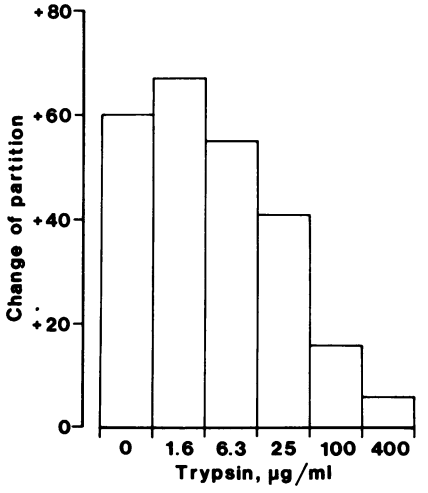

FIG. 1. Effect of trypsin digestion of a group A streptococcus (strain AM23) on the CP in polymer two-phase systems with and without 0.25% St-PEG. Bacteria were pretreated with various concentrations of trypsin as indicated. For phase composition and calculation of CP see the text.

Enzymatic digestion and heat treatment of group A streptococci. Bacteria were treated with trypsin (Sigma Chemical Co.; catalog no. T-8253) by suspending 5.0×10^8 organisms in 0.5 ml of PBS containing 150 μg of trypsin per ml. In some experiments, increasing amounts of trypsin were added to the cells. After 45 min of incubation at 37°C, the tryptic activity was stopped by the addition of 0.5 mg of trypsin inhibitor (Sigma Chemical Co.; catalog no. T-9128) and by subsequent washings in PBS. Organisms were treated with pepsin (Sigma Chemical Co.; catalog no. P-7012) by washing them once in 0.1 M acetate buffer, pH 4.5 or in 0.1 M phosphate buffer, pH 5.8, and resuspending 5.0×10^8 bacteria in 0.5 ml of the respective buffer containing 100 μg of pepsin per ml. The mixture was incubated at 37°C for 30 min, and the reaction was terminated by the addition of 100 μl of 1 M Tris base. Treatment with papain (Sigma Chemical Co.; catalog no. P-9886) was performed by resuspending 5.0×10^8 bacteria in 0.5 ml of 0.1 M Tris base–0.02 M cysteine (pH 8.0) containing 200 μg of papain per ml. The mixture was incubated at 37°C for 45 min. The streptococci were also treated with hyaluronidase (Sigma Chemical Co.; catalog no. H-3506) by resuspending 5.0×10^8 bacteria in 0.5 ml of PBS containing 50 U of hyaluronidase per ml followed by incubation at 37°C for 60 min. Heat treatment of streptococci suspended in PBS was performed at 100°C for 10 min. After enzymatic and heat treatment, the bacteria were washed twice in ice-cold PBS and resuspended in appropriate solutions for use in polymer two-phase systems or LTA assays.

Polymer two-phase system. PEG 4000 was obtained from Union Carbide Corp., New York, N.Y., and dextran T500 (lot no. FD 16027) from Pharmacia Fine Chemicals, Inc., Uppsala, Sweden. Stearate PEG (St-PEG) was purchased from Serva, Heidelberg, Federal

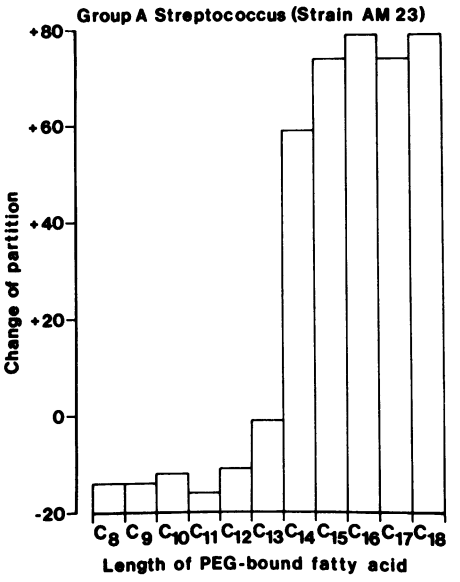

FIG. 2. Effect of PEG-bound fatty acids of various carbon chain lengths on the partition of a group A streptococcus (strain AM23) in polymer two-phase systems. PEG esters (0.5%) were used. For phase composition and calculation of CP see the text.

Republic of Germany. PEG esters (C_8 to C_{18} fatty acid esters of PEG 6000) were prepared with dicyclohexylcarbodiimide mainly according to Johansson (15), but without copper(I) iodide as a catalyst. Two-phase systems were prepared from stock solutions of dextran (20%), PEG 4000 (40%), and PEG esters (2.5 to 5.0%). Salt and buffer stock solutions were made up to concentrations 10 to 20 times higher than that of the final concentration in the phase system.

Hydrophobic affinity partition experiments. Single-tube partitions were performed in plastic tubes (70 by 11 mm; A/S NUNC, Roskilde, Denmark) with 1.00-g-weight phase systems. The phase systems were composed of 6.1% (wt/wt) dextran, 6.1% (wt/wt) PEG, 5 mM sodium phosphate buffer (pH 6.9), 40 mM NaCl, and 0 to 0.5% (wt/wt) PEG esters. A batch system was prepared from stock solutions of dextran, PEG 4000, sodium phosphate buffer, and sodium chloride. A 0.80-g sample was taken from the batch system immediately after mixing. Adding 100 μl of esterified PEG solution or sterile water and 100 μl of bacteria in sterile water gave the desired final concentration of all components. The phase systems were mixed thoroughly by inverting the tubes about 50 times. After a 40-min settling time, 100 μl of the top and bottom phases was withdrawn from each tube with a calibrated pipette (Oxford Laboratories, Foster City, Calif.). The radioactivity in each sample was measured in a gamma counter (LKB-Wallac 1270 Rackgamma; LKB Sverige AB, Bromma, Sweden). In experiments with beta emitters, the samples were collected on glass microfiber filters (2.5 cm; Whatman Ltd., Maidstone, England). Dried filters were placed in scintillation vials, and 7.5 ml of scintillation fluid [5 g of PPO (2,5-diphenyloxazole) and 0.1 g of dimethyl 1,4-bis-(5-phenyloxazolyl)benzene (Packard Instrument Co., Inc., Rockville, Md.) dissolved in 1 liter of toluene (E. Merck AG, Darmstadt, Germany)] was added to each vial. The samples were counted in an Intertechnique SL 4000 scintillation counter (Plaisir, France). Phase systems were prepared, and experiments were carried out at room temperature. All experiments were performed in duplicate.

Presentation of partition data. The percent bacterial cells in the top and bottom phases was calculated from the radioactivity measured. The results are presented in the present studies as change of partition (CP) as described by Magnusson et al. (19). CP was calculated using the following formula: $CP = (T_{F\text{-}PEG} - B_{F\text{-}PEG}) - (T_{basal} - B_{basal})$ where T is the percent bacteria in the PEG-rich top phase and B is the percent bacteria in the dextran-rich bottom phase. F-PEG indicates the phase system containing PEG-bound fatty acid, and basal indicates the phase system without hydrophobic groups. The value of CP can vary in the range 0 to 200 and is positive when hydrophobic partitioning (i.e., transfer from the lower phase to the interface or from the interface to the upper phase) is involved. A negative value of CP means that the cells are excluded from the upper phase when the fatty acid group is introduced.

LTA assay. A rabbit antiserum raised against *Lactobacillus casei* LTA (kindly provided by K. W. Knox, Sydney, Australia) was used for determining cell wall LTAs on whole organisms. Staphylococcal protein A

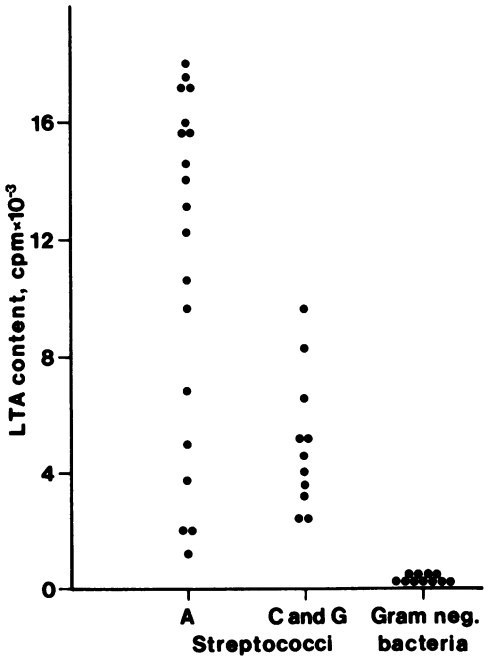

FIG. 3. Determination of surface LTA in bacterial strains. Group A, C, and G streptococci and gram-negative bacteria were labeled with antibodies to LTA and treated with ^{125}I-labeled protein A. The data are presented as corrected counts per minute (counts per minute with antibodies to LTA minus counts per minute with control consisting of normal rabbit serum).

TABLE 2. Effects of enzymatic treatment of group A streptococci on the binding of antibodies to LTA to their surfaces

Bacterial strain	Antibody binding (cpm × 10^3)					
	PBS control	Trypsin[a]	Pepsin[b]		Papain[c]	Hyaluronidase[d]
			pH 4.5	pH 5.8		
AW4	7.1	1.4	4.5	5.5	4.6	6.1
AM1	9.6	2.0	4.2	7.0	4.1	8.9
AM18	7.9	1.7	3.8	6.3	3.5	6.7
AM23	9.2	1.3	2.5	9.5	2.6	9.4

[a] A total of 150 μg of trypsin per ml, pH 7.2.
[b] A total of 100 μg of pepsin per ml.
[c] A total of 200 μg of papain per ml, pH 8.0.
[d] A total of 50 U of hyaluronidase per ml, pH 7.2.

(Pharmacia Fine Chemicals) was labeled with ^{125}I by the chloramine-T method (22) and used as an antiantibody reagent (12). The LTA assay was performed in duplicate in plastic tubes (70 by 12 mm; AB Cerbo, Trollhättan, Sweden). A 50-μl amount of bacterial suspension (5.0×10^7) in PBS was added to 100 μl of serum (LTA antiserum and, as a control, normal rabbit serum) diluted 1/100 in PBS containing 0.1% bovine serum albumin (PBS-BSA). After incubation at 37°C for 40 min, 1.5 ml of PBS-BSA was added to each tube, and the bacteria were deposited by centrifugation. Supernatants were removed, and 100 μl of ^{125}I-labeled protein A was added to each tube. The mixture was incubated for another 40 min at 37°C, washed one time in PBS-BSA, and centrifuged. Supernatants were discarded, and the radioactivity of the bacterial pellets was measured in a gamma counter (LKB-Wallac 1260 Multigamma; LKB Sverige AB).

Extraction of LTA from streptococci. Group A streptococcus strain AM23 was grown overnight in 100 ml of Todd-Hewitt broth supplemented with 150 μCi of tritiated glycerol (specific activity, 500 mCi/mmol; Radiochemical Centre). The bacteria were harvested and washed five times in distilled water. Extraction was performed on 0.2 g (wet-packed weight) of bacteria suspended in 0.5 ml of distilled water with an equal volume of 90% (wt/vol) phenol at 65°C (42, 44). After being mixed for 5 min, the suspension was centrifuged at 12,000 × *g* for 30 min. The aqueous phase was removed and dialyzed against six changes of distilled water for 3 days. The presence of LTA in the extract was demonstrated by precipitation with an antiserum against *L. casei* LTA. A 50-μl amount of LTA antiserum and 25 μl of tritiated aqueous phenol extract were mixed and diluted with 175 μl of PBS. Normal rabbit serum was used as a control. After incubation overnight at 37°C, 150 μl of bovine serum and 800 μl of 15% PEG 6000 were added to each tube. The mixture was centrifuged at 1,000 × *g* for 45 min. The pellet was dissolved in scintillation fluid (Biofluor; New England Nuclear Corp.), and the radioactivity was measured in a beta scintillation counter.

RESULTS

Partition of bacteria after enzymatic and heat treatment. The differential effect of proteolytic digestion on bacterial surface structures was utilized to characterize the hydrophobic factor on the surface of group A streptococci. Four proteolytically modified group A streptococcal strains were partitioned in polymer two-phase systems. Bacteria (5.0×10^8) were suspended in 0.5 ml of enzyme solutions and incubated at 37°C for 30 to 60 min before partition experiments. Trypsin digestion (150 μg/ml) had a marked effect on the partition of the bacteria (Table 1). Trypsin-treated bacteria lost their hydrophobic character. The addition of increasing amounts of trypsin to group A streptococcus strain AM23 showed that this effect was dose dependent (Fig. 1). Treatment of group A streptococci with pepsin (100 μg/ml) at pH 4.5 or papin (200 μg/ml) also resulted in a marked

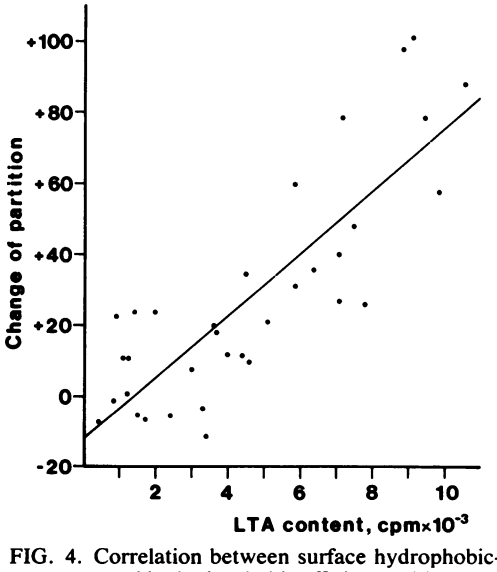

FIG. 4. Correlation between surface hydrophobicity, as measured by hydrophobic affinity partition, and the content of surface LTA. The CP of 34 group A streptococcal strains is plotted against the content of surface LTA expressed as counts per minute (r = 0.84). The two-phase systems used are the same as in Fig. 1.

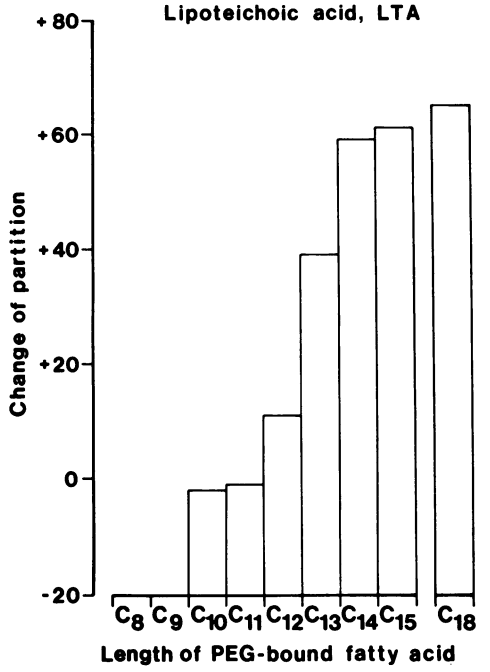

FIG. 5. Effect of PEG-bound fatty acids of varying length on the partition of ^{3}H-labeled LTA in polymer two-phase systems. For phase composition and calculation of CP see the text.

decrease in CP (Table 1). Digestion with pepsin (100 μg/ml) at the suboptimal pH 5.8 had, however, no significant effect on the partition of the bacteria in polymer two-phase systems (Table 1). Bacterial cells treated with 50 U of hyaluronidase per ml, in most instances, showed a slight increase in CP as compared with the control (Table 1). Heat treatment (100°C for 10 min) did not influence the partitioning of the cells (Table 1).

Effect of PEG-bound fatty acids of various lengths on the partition of group A streptococci. The length of the PEG-bound fatty acid influences the accessibility to the hydrophobic part of the molecules investigated (15, 16). Three group A streptococcal strains were partitioned in phase systems with PEG-bound fatty acids of various lengths. The final concentration of PEG-bound fatty acids in the phase systems was 0.5% (wt/wt). PEG-bound fatty acids C_8 to C_{12} had a negative effect on the CP (i.e., an increase in the affinity of the bacteria for the dextran-rich bottom phase), whereas C_{14} (myristic acid) to C_{18} (stearic acid) fatty acids markedly increased the affinity of the bacteria for the interface and the upper phase (Fig. 2). Similar effects have been observed when serum albumin is partitioned in this type of two-phase system (15).

Determination of surface LTA. Cell wall teichoic acids were measured on whole bacterial organisms in a radioimmunoassay utilizing a rabbit antiserum against LTA and ^{125}I-labeled protein A as a second-layer antiantibody. Normal rabbit serum was used as a control. The binding of anti-LTA to group A, C, and G streptococci varied substantially, whereas none of the gram-negative bacteria tested bound any anti-LTA (Fig. 3). Four group A streptococcal strains were treated with proteolytic enzymes before LTA analysis. Trypsin, pepsin (pH 4.5), and papain treatment of the bacteria had a marked effect on the binding of anti-LTA to the bacterial surfaces (Table 2). Pretreatment of bacteria with pepsin at suboptimal pH 5.8 and with hyaluronidase had no significant effect on the binding of anti-LTA to the bacteria (Table 2).

To elucidate the relation between surface LTA and the hydrophobicity of the bacterial cells, 34 group A streptococcal strains were tested in the LTA assay and in hydrophobic affinity partition experiments. The results are presented in Fig. 4 and show a correlation between the binding of anti-LTA and the CP in polymer two-phase systems.

Partition of LTA in polymer two-phase systems. Group A streptococcus strain AM23 was labeled with tritiated glycerol. The LTA of the bacterial preparation was extracted by hot aqueous phenol and tested in polymer two-phase systems containing PEG-bound fatty acids of varying length. As shown in Fig. 5, fatty acids with less than 13 carbon atoms had no significant effect on the partition of LTA, whereas the longer fatty acid chains markedly increased the affinity of the extracted LTA for the upper phase. The high affinity of radiolabeled LTA for St-PEG was retained after treatment of the LTA extract with trypsin (200 μg/ml) or papain (200 μg/ml) for 45 min at 37°C.

Partition of bacteria labeled with different radioisotopes. The influence of different radiolabeling procedures on the hydrophobic affinity partition of bacteria was investigated. Todd-Hewitt broth supplemented with [^{3}H]thymidine (2 μCi/ml) was inoculated with a group A streptococcus (strain AM23) and incubated overnight. After washing in PBS, one portion of the bacteria was labeled with ^{51}Cr, another portion was labeled with ^{125}I, and the third portion received no further treatment. After radiolabeling and subsequent washing, the partition of the bacteria was studied in polymer two-phase systems. Bacteria labeled with different radioisotopes behaved similarly when partitioned with various concentrations of St-PEG (Fig. 6). In the phase system without St-PEG, the ^{51}Cr- and ^{125}I-labeled bacteria partitioned more in favor of the top phase as compared with [^{3}H]thymidine-labeled cells. Control experiments showed that

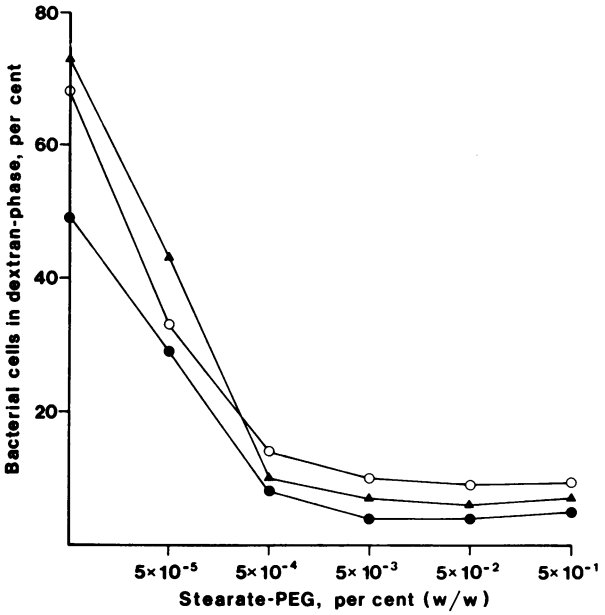

FIG. 6. Effect of different labeling procedures on the partition of a group A streptococcus (strain AM23) in a polymer two-phase system at increasing St-PEG concentrations. The data are presented as the percent bacterial cells in the dextran-rich bottom phase. (For phase composition see the text.) Symbols: bacteria labeled with [^{3}H]thymidine (●), ^{125}I (○), and ^{51}Cr (▲).

this difference was due to the physicochemical conditions of the labeling procedure (i.e., prolonged incubation at 37°C, lactoperoxidase, and hydrogen peroxide) rather than the radioactive isotope itself. All experiments were performed in triplicate. The standard error of the mean never exceeded 6%.

DISCUSSION

It has previously been suggested that M protein of group A streptococci is responsible for the hydrophobicity of these microorganisms (24, 35, 36). M protein has been claimed to mediate the binding of group A streptococci to oral epithelial cells (9). Since hydrophobicity of microorganisms is considered a major determinant for adherence to host cells (20, 28, 32, 38, 39), the importance of M protein therefore seems probable. However, in more recent studies, no significant differences in adherence were found between M-positive and M-negative strains of group A streptococci (3, 8). The removal of M protein by mild pepsin digestion did not abolish the adhering ability of streptococci (8). In the present studies, experiments were performed aimed at defining surface structures responsible for the hydrophobic character of the bacteria.

Bacteria were treated with various proteolytic enzymes with differential effects on surface structures of streptococci. Digestion with trypsin, papain, and pepsin all resulted in a decrease in hydrophobic affinity of the bacteria (Table 1 and Fig. 1). Mild pepsin digestion at pH 5.8, which is known to cleave the distal portion of the M protein molecule (29), had no significant effect on bacterial surface hydrophobicity (Table 1). These results indicate that the hydrophobic surface structure on group A streptococci either consists of a protein or molecules linked to proteins. Protein solubilized from streptococci by proteolytic digestion often contains LTA (17). Mild pepsin digestion of group A streptococci at a suboptimal pH removes M protein, whereas LTA is retained on the surface (8).

The role of LTA in conferring hydrophobicity was elucidated by assessing the quantity of surface LTAs in a double-layer radioimmunoassay. Antibodies to LTA bound at varying degrees to all group A, C, and G streptococci (Fig. 3). Enzymatic digestion of bacteria before LTA assay revealed that surface LTA was partially removed after treatment with trypsin, papain, and pepsin (Table 2). Mild pepsin digestion had no significant effect on the content of surface LTA (Table 2). This indicates that the surface LTA molecule is bound to a cell wall protein. The nature of the surface protein to which LTA is anchored is unknown. The possibility still remains that LTA binds to a portion of M protein near the surface of the bacteria.

A direct comparison between surface hydrophobicity and LTA was made. The quantity of surface LTA and the degree of surface hydro-

phobicity were determined on a collection of group A streptococci. The results show a correlation between the hydrophobic affinity partition of the bacteria in polymer two-phase systems and the quantity of surface LTA (Fig. 4), indicating that LTA plays a major role in conferring hydrophobicity to group A streptococci. However, the possibility that other surface molecules also contribute to the hydrophobicity of group A streptococci cannot be ruled out.

In further experiments on hydrophobic surface structures, LTA was extracted from intact group A streptococci by the hot aqueous phenol method (42, 44). This extraction procedure yields a preparation of low protein content (44). The LTA extract showed a high affinity for St-PEG when tested in two-phase systems. Treatment of LTA extract with papain, which is known to further lower the protein content of the extract (44), did not influence the hydrophobic affinity of LTA. These results indicate that the hydrophobic affinity expressed by the LTA extract is due to LTA itself rather than a protein bound to LTA.

The length of PEG-bound fatty acids used in the test determines the liability to hydrophobic interactions in polymer two-phase systems (10, 16). Experiments were performed with fatty acids with lengths of 8 to 18 carbon atoms. The partition of whole group A streptococci as well as solubilized LTA required a fatty acid chain of 14 to 13 carbon atoms, respectively, in order to be transferred from the bottom phase into the interface and top phase (Fig. 2 and 5). This similarity in partition behavior between LTA and intact organisms indicates that LTA in situ is a major factor responsible for the hydrophobic surface properties of group A streptococci. The difference of one carbon atom indicates that the cell-bound LTA is somewhat restricted in its interaction with PEG-bound fatty acid.

The hyaluronate capsule might mask hydrophobic properties of group A streptococci. Enzymatic removal of hyaluronic acid from bacteria harvested during the stationary phase had no marked effect on either hydrophobic affinity partition or surface LTA (Tables 1 and 2). Hyaluronic acid is known to accumulate on the bacterial surface during logarithmic growth followed by a decrease when the bacteria enter the stationary phase (30). Bacteria were therefore also harvested during the logarithmic growth phase and tested in polymer two-phase systems. Bacteria from these cultures partitioned in a similar way in polymer two-phase systems, indicating that hyaluronic acid has no marked influence upon surface hydrophobicity.

Studies of *Salmonella typhimurium* have revealed that phagocytosis-sensitive R mutants are liable to hydrophobic and ionic interactions, whereas the smooth phagocytosis-resistant strains have a hydrophilic and uncharged surface (20). The adherence of *E. coli* correlates to their degree of hydrophobicity (28, 32). The importance of hydrophobicity for the phagocytosis of bacteria has been demonstrated by Van Oss (38). Group A streptococci are negatively charged in a physiological milieu (13, 24). Most mammalian cells also carry a net negative charge. LTA on the cell surface of group A streptococci makes these cells markedly hydrophobic. The liability to hydrophobic interactions might serve to overcome the electrical repulsion and thereby facilitate the initial steps in adherence.

A variety of biological properties have been ascribed to LTA. It has been suggested to play a central role in the pathogenesis of streptococcal infections (18, 26, 46). LTA is known to bind to epithelial cells (4, 26), erythrocytes (7, 46), phagocytes (25), and human platelets (5). Hypersensitivity reactions and suppression of the immune response in laboratory animals have been demonstrated to be affected by LTA (14, 23). LTA also acts as a nonspecific T-lymphocyte mitogen (6). The lipid portion of LTA has proved to be essential for biological properties such as immunogenicity (45) and binding of LTA to cell membranes (26). In addition to these properties, the present investigation indicates that LTA is a major hydrophobic constituent of group A streptococci.

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